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Low frequency of pre-treatment and post-treatment haematological abnormalities in dogs with non-infectious meningoencephalitis treated with cytosine arabinoside and prednisolone

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ABSTRACT

Background Cytosine arabinoside (CA) and prednisolone are drugs commonly used together in the management of canine non-infectious meningoencephalitis (NIME). The aim of this study was to report the haematological findings before and after CA and prednisolone treatment and identify any adverse haematological events in this clinical setting, following the veterinary cooperative oncology group established common terminology criteria for recording adverse events following administration of chemotherapy or biological antineoplastic therapy.

Results While 48 patients with a presumptive diagnosis of NIME had pretreatment haematology results, only 12 patients met the inclusion criteria of also having post-treatment haematology results available for review after being treated with prednisolone and CA at a standard dose (200 mg/m²) in a single referral hospital in the UK. Forty-nine post-treatment haematology results were available for these 12 patients.

Conclusions Four adverse haematological events were identified in four patients. None of these events were convincingly attributable to CA administration.

INTRODUCTION

Non-infectious meningoencephalitis (NIME) is a term used to characterise inflammatory brain diseases for which no underlying infectious agent is identified and when an immune-mediated basis is suspected.^{1–4} The terms meningoencephalitis of unknown origin (MUO)³ or meningoencephalitis of unknown aetiology are also commonly used as the pathophysiology of NIME is still poorly understood.⁵ A histopathological diagnosis is necessary to reach a definitive diagnosis (eg, granulomatous meningoencephalitis, necrotising leukoencephalitis or necrotising meningoencephalitis); however, a presumptive diagnosis is often made based on signalment, neurological examination, advanced imaging, cerebrospinal fluid analysis and

infectious disease testing.^{1 2 5–9} There remains considerable debate regarding what constitutes the preferred treatment for cases diagnosed with NIME.^{3 10–14} Treatment is centred on immunosuppression and while multiple protocols are available, few comparative studies have been published.³ Prednisolone monotherapy and cytosine arabinoside (CA) alongside prednisolone are two of the immunosuppressive protocols of choice for the treatment of NIME,^{10 13} with one recent study suggesting that early treatment with a constant rate infusion (CRI) of CA could reduce the early mortality associated with the disease.¹⁴ CA, an S-phase, cell cycle-specific cytotoxic agent, is converted on entering cells to the active form arabinosylcytosine triphosphate. Several mechanisms of action have been proposed to explain its cytotoxic activity including inhibition of DNA polymerase α and incorporation into the DNA strand. The overall result being inhibition of DNA synthesis.¹⁵ As a cytotoxic drug, CA exerts its effects on all proliferating cells, such as the haematopoietic progenitor cells in the bone marrow, and this can lead to myelosuppression. In dogs, the time to maximal myelosuppression (nadir) following CA administration alone is considered to be close to 8–10 days after treatment.¹⁶

There are limited reports in the literature regarding adverse effects; however, anecdotally, there would appear to be very few adverse events when using CA. Potential adverse reactions reported in association with CA use include dermatological disease following subcutaneous injections in dogs treated for NIME^{17 18} and infiltrative lung disease.¹⁹ The incidence of myelosuppression

following CA administration for the treatment of canine lymphoma (600 mg/m^2 intravenously over 48 hours) has been assessed in a previous publication, and their results showed that 10 of 14 patients developed at least one cytopenia.²⁰ While the risk of haematological adverse events following the administration of prednisolone and CA at the dose of 200 mg/m^2 seems to be very low, few clinical studies have reported on the safety of this protocol in the treatment of NIME.^{10 14 21} In those studies, pretreatment haematology results were not reported in detail, and the post-treatment haematology was either performed three weeks after CA administration,¹⁴ was not performed in all dogs²¹ or was not described in enough detail to document potential side effects.¹⁰ More recently, a study evaluating the pharmacokinetics of CA alone in a population of healthy animals used at 200 mg/m^2 (either over 48 hours as subcutaneous injections or over eight hours as an intravenous CRI) reported that all six patients included in the study had haematological parameters within normal limits before treatment and seven days after CA administration.²²

The aim of our study was to estimate the prevalence of pretreatment and post-treatment haematological abnormalities in patients with a presumptive diagnosis of NIME treated with a combination of prednisolone and CA.

METHODS

Study design

This was a retrospective, descriptive, single-centre study performed at a university referral hospital in the UK between 2010 and 2016. All patients were client-owned dogs. The clinical database, client and referring veterinarian communications were searched for patients who received CA between 2010 and 2016.

Inclusion and exclusion criteria

To be included in this study, patients had to have been referred to the hospital with neurological signs and have undergone investigations leading to a presumptive diagnosis of NIME made by a board-certified neurologist or a resident working under their guidance. A presumptive diagnosis of NIME was reached based on patients fulfilling the criteria set out in previous publications including neurological examination findings, MRI or CT findings, infectious diseases testing and, when safe, cerebrospinal fluid analysis (CSF).³ All patients had to have received corticosteroids and CA as part of their treatment protocol for NIME. The total CA dose administered for each treatment had to be 200 mg/m^2 . This had to be given either as an intravenous CRI given over 8–24 hours, or via four 50 mg/m^2 subcutaneous injections at 12-hour intervals. Data regarding age, sex, breed, pretreatment and post-treatment haematology results were obtained. A complete pretreatment haematology performed in the four weeks prior to CA administration had to be available for review. However, only the haematocrit or packed cell volume, neutrophil count and platelet count were

recorded and examined for the purpose of the study, using a similar approach evaluated in a previous study.²⁰ A platelet count that fell below the machine reference interval had to be confirmed with a manual blood film evaluation and platelet count. Additionally, the date of CA administration and the date of post-treatment haematology assessment was recorded. All post-treatment haematology assessments had to be performed 5–14 days following CA administration. The dose in mg/kg of prednisolone was recorded at each haematological assessment. Haematology assessments were performed either at the referral hospital, an external laboratory or at the referring veterinary clinic. Reference ranges were therefore not all the same.

Exclusion criteria included: any patient receiving a drug other than CA that was known to have a direct myelosuppressive effect; any patient receiving a different dose of CA; any patient for whom the pretreatment or post-treatment haematology results could not be reviewed. Patients receiving drugs reported to cause idiosyncratic haematological abnormalities (eg, phenobarbitone or trimethoprim-sulphonamide) were not excluded.

Grading of adverse effects

Each post-treatment haematology report was considered an event. Each event was individually graded using the established veterinary cooperative oncology group - common terminology criteria for adverse events (VCOG-CTCAE) haematological grading system.²³

Results are reported as frequencies using numbers and percentages with 95 per cent confidence interval (CI) where appropriate.

RESULTS

Seventy-four patients were initially identified. Sixty-two patients were excluded: 19 cases did not have MUO as their final diagnosis. Of the remaining 55 cases, 48 had a pretreatment haematology available for review (online supplementary file 1). Only 12 cases received the standard dose of 200 mg/m^2 of CA and had a pretreatment and post-treatment haematology available for review (online supplementary file 2).

Of these 12 cases, the following breeds were included: two Chihuahuas, two West Highland white terriers, two shih tzus, two labradors, a Maltese, a bull terrier, a whippet and an Airedale terrier. Median age was 77.5 months (range 10–131 months). Neutering status included: seven neutered females, three neutered males, one entire male and one entire female (table 1).

Haematology results at diagnosis

Forty-eight patients with a diagnosis of NIME that subsequently received CA administration had a haematology at diagnosis available for review (online supplementary file 1). In six patients (12.5 per cent, 95 per cent CI 5 to 26), the haematocrit was outside the reference range. Anaemia was recorded in four patients and erythrocytosis in two patients. Although 28 patients (58 per cent,

Table 1 Descriptive data for the 12 patients undergoing therapy with a standard dose of cytosine arabinoside, including concurrent therapy over the course of treatment

Patient number	Age at diagnosis (months)	Sex	Breed	Concurrent drugs
1	72	FN	Chihuahua	Prednisolone, trimethoprim-sulphonamide, omeprazole, sucralfate, clindamycin and topical chloramphenicol eye-drops.
2	131	MN	West Highland white terrier	Prednisolone, omeprazole, levetiracetam, topical miconazole nitrate, prednisolone acetate and polymyxin B sulfate.
3	61	FN	shih tzu	Prednisolone, cyclosporine and cephalexin.
4	52	FN	Maltese	Prednisolone and cyclosporine.
5	16	ME	Chihuahua	Prednisolone, doxycycline, phenobarbitone, levetiracetam and cyclosporine.
6	112	FN	shih tzu	Prednisolone.
7	96	MN	West Highland white terrier	Prednisolone, cephalexin, theophylline and topical fusidic acid.
8	57	FN	whippet	Prednisolone, phenobarbitone, potentiated amoxicillin, metronidazole, sucralfate and cimetidine.
9	119	FN	Airedale	Prednisolone.
10	83	FN	Labrador	Prednisolone, phenobarbitone, potentiated amoxicillin, omeprazole, sucralfate and maropitant.
11	10	FE	Labrador	Prednisolone, potentiated amoxicillin and omeprazole.
12	115	MN	bull terrier	Prednisolone.

FE, female entire; FN, female neutered; ME, male entire; MN, male neutered.

95 per cent CI 9 to 33) had an abnormality noted on their leucogram, only nine patients (18.8 per cent, 95 per cent CI 43 to 72) had either a leucocytosis (5/9) or leucopenia (4/9) reported. Six patients (12.5 per cent, 95 per cent CI 5 to 26) were reported to have either a thrombocytosis (5/6) or thrombocytopenia (1/6). In the single patient with a thrombocytopenia on the machine count, no blood smear or manual platelet count were performed.

Post-treatment haematology results

Twelve patients included in the study received a total of 49 standard CA treatments for which the post-treatment haematology reports were available for review (online supplementary file 2). The median time from CA administration to post-treatment haematology assessment was 7 days (range 6–13 days). Nine (18 per cent) of these 49 treatments were administered as a CA intravenous CRI given over a period between 8 hours and 24 hours. The remaining 42 (82 per cent) treatments were given as subcutaneous injections of CA over 48 hours. Of the 49 post-treatment haematology results available to review, we identified four (8 per cent, 95 per cent CI 3 to 21) instances of an adverse haematological event in four patients (33 per cent, 95 per cent CI 11 to 65), and this was anaemia in each instance (online supplementary file 3), as determined by the VCGO-CTCAE haematological toxicity grading system (online supplementary file 2). Patient 3 who had been noted to have a borderline non-regenerative anaemia prior to treatment was documented to have a persistent mild non-regenerative anaemia following their first CA treatment equating to

VCGO-CTCAE grade 1. Patient 4 was noted to develop a mild anaemia equating to VCGO-CTCAE grade 1 following their first CA treatment. Patient 9 developed a moderate anaemia which was markedly regenerative and a thrombocytosis following their third CA treatment equating to VCGO-CTCAE grade 2. Patient 11 developed a mild non-regenerative anaemia equating to VCGO-CTCAE grade 1 following their first CA treatment. Taking these factors into consideration, a total of four haematological abnormalities were identified over 49 events. Prednisolone dose and concurrent medications were recorded for each patient at the time of the haematology assessment (online supplementary file 2).

DISCUSSION

The incidence of haematological abnormalities in patients with a presumptive diagnosis of NIME is sparsely documented in the current veterinary literature.^{35–810–1424} A unique aspect of this study is the review of the haematological findings at diagnosis of a group of patients with presumptive NIME subsequently treated with prednisolone and a standard dose of CA. While abnormalities of the leukogram at diagnosis were not uncommon in our patient population, some of the changes could be explained by the recent administration of corticosteroids either at anti-inflammatory or immunosuppressive doses (4/8 cases presented with a neutrophilia were receiving corticosteroids at the time of haematological testing; online supplementary file 1).

Administration of cytotoxic agents comes with the risk of adverse events particularly haematological toxicity.

Assessment of 49 post-treatment haematology reports identified four haematological abnormalities following CA administration.

The four adverse events included a mild anaemia in three patients after their first CA treatment and a moderate anaemia in one patient following their third CA treatment. In all cases, confounding factors meant that the anaemia could not be definitively attributed to CA administration. Confounding factors included suspected gastrointestinal bleeding and, in one patient, exploratory laparotomy. In each case, the mild-to-moderate anaemia although not directly attributable to CA treatment may have had a slower resolution due to the myelosuppressive effect of CA on erythropoiesis. Three of these four cases were patients who had received intravenous CA administration. The significance of this finding is unknown and could be the grounds for a future prospective study.

There are limitations to this study, due to its retrospective nature and due to the inclusion criteria resulting in a small number of patients being eligible for review. Additionally, ideally, we would have evaluated a population of canine patients diagnosed with NIME receiving only CA and prednisolone. However, given the retrospective nature of our study this was not possible. Our exclusion criteria dictated that patients receiving drugs known to have myelosuppressive effects were excluded. Ultimately, no patients were excluded due to this criterion; however, all patients received additional drugs, for example, anaesthetic agents or intravenous fluids in the course of their investigations and treatment. Many patients in our population also required adjunctive therapy in the management of their concurrent clinical signs, for example, omeprazole or antiseizure medication, for example, phenobarbitone, the latter of which has been reported to cause idiosyncratic reactions. As we had a plausible explanation for all post-treatment haematological abnormalities identified, we did not consider that concurrent drug administration was a contributing factor to any post-treatment haematological abnormalities.

As outlined above, our results support that the myelosuppressive effect of CA at this dose appears low. Being a retrospective study, we could not standardise the laboratories used, resulting in some haematology testing being performed in-house, while others were performed in external laboratories. As age, sex and breed are known to affect haematological parameters and given the small and diverse patient population in our study, any statistical comparison was considered inappropriate.^{25–28} A larger prospective study with rigorous standardisation of the timing and methodology of haematological assessment following CA administration would provide a more accurate quantification of the adverse event rate. All post-treatment haematology assessments in our study were performed between 6 days and 12 days following CA administration, that is, close to the expected nadir. However, a limitation resides in the fact that the post-treatment haematology assessment may have missed the nadir for some patients leading to a false negative

result for an adverse haematological event. Lastly, we did consider the possibility of including a control group of NIME patients treated with prednisolone only. However, patients treated with corticosteroid monotherapy do not have routine haematology performed during their treatment; therefore, it was not possible to retrospectively provide a matched control group. This could be considered if a future prospective study was designed.

Our small study population reflects our inclusion and exclusion criteria and limits the conclusions we can draw. We concluded that routine post-treatment haematology assessment following administration of 200 mg/m² CA in dogs identified four adverse haematological events, two of which were attributed to prednisolone induced gastrointestinal bleeding. Further studies would be needed to determine if our findings can be extrapolated to a larger population of NIME patients receiving CA and prednisolone therapy.

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Competing interests None declared.

Ethics approval Ethical approval was obtained from the ethics board of the university (VIN/16/054).

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Data sharing statement All data relevant to the study are included in the article or uploaded as supplementary information.

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